**Section I. (Amendments to the Specification)**

Please amend the specification as follows:

At page 2 of the specification, please replace the paragraph beginning at line 8 with the following new replacement paragraph:

> These limitations in the manipulation and generation of the different *in vitro* cell ~~cultures~~ models make them difficult to implement for occasional users, and in general limit the marketing of the models in their end format. The problem gets worse in complex models, wherein the cells should imitate the organism's natural barriers (Rubas et al., J. Pharma. Sci., 85:165-169, 1996; Walter et al., J. Pharma Sci., 85:1070-1076; Irvine et al., J. Pharma Sci., [[88:28:28-33]] 88:28-33, 1999; Gaillard et al,. Eur. J. of Pharma Sci., 12:215-222, 2001); or the cultures should be performed on asymmetric supports, separating two compartments or with a strong dependence on the polarisation of the system components. In these cases, to the complexity of the model and its time limits, we can add mechanical-type problems, which mean bumps or shaking may invalidate the system. Researchers can access the different model components (support, culture medium and additives and cell lines) and later they should combine them in the laboratory using more or less laborious processes). In the best of the cases, the final researcher may receive the cells ready-for-use, but with limitations which practically mean it is obligatory to perform the experiments within two days after their reception, and they impose serious restrictions on the distribution of the model by the company marketing it, such as, for example, In Vitro Technologies. Document EP 702 081 discloses a method for the storage and transport of three-dimensional tissues which consists of positioning said three-dimensional tissue fixed on two types of sponges in a gelatine solution, so that this gels with cooling, thus making it easier to transport and store.

At page 3 of the specification, please replace the paragraph beginning at line 3 with the following replacement paragraph:

Therefore, there is an existing need in the state of the art for providing a method to be able to supply models based on ready-to-use organized two-dimensional cell cultures and with their functional properties intact so that, on the one hand, the researcher has a margin of manoeuvre for its use and, on the other, the supplying company may consider delivery times within the reasonable logistic margins of international distribution.

At page 3 of the specification, please replace the paragraph beginning at line 11 with the following replacement paragraph:

The object of the present invention consists of providing a method of storing and transporting *in vitro* organized two-dimensional cell cultures which resolves the aforementioned needs of the state of the art.

At page 3 of the specification, please replace the paragraph beginning at line 24 with the following replacement paragraph:

In its main aspect, the invention provides a method of storing and/or transporting *in vitro* organized cell cultures which comprises the following steps:

a) coating [[a]] an organized cell culture that is immobilised on an asymmetric support with a gelatine solution in the culture medium at a concentration of between 1 and 5 %, said cell culture comprising cells in suitable functional state,

b) solidifying the gelatine added to the support at a temperature of between 15 and 25°C, and

c) storing and/or transporting the cell culture at a temperature of between 15 and 25°C for a period of up to 96 hours.

At page 3 of the specification, please replace the paragraph beginning at line 36 with the following replacement paragraph:

In a second aspect, [[The]]the present application also ~~relates to~~ provides a kit ~~which is used to store and/or transport~~ for storing and/or transporting the *in vitro* two-dimensional cell cultures according to the method of the invention which

comprises:

(i) an asymmetric support, and

(ii) a gelatine solution in the culture medium at a concentration of between 1 and 5%.

At page 4 of the specification, please replace the paragraph beginning at line 8 with the following replacement paragraph:

In its main aspect, the invention provides a method of storing and/or transporting *in vitro* organized two-dimensional cell cultures which comprises the following steps:

a) coating [[a]]an organized cell culture that is immobilised on an asymmetric support with a gelatine solution in the culture medium at a concentration of between 1 and 5 %, said cell culture comprising cells in suitable functional state,

b) solidifying the gelatine added to the support at a temperature of between 15 and 25°C, and

c) storing and/or transporting the cell culture at a temperature of between 15 and 25°C for a period of up to 96 hours.

At page 5 of the specification, please replace the paragraph beginning at line 1 with the following replacement paragraph:

The two-dimensional cell cultures of the invention are organised cultures such as, for example: Huvec cells, grown to confluence on a collagen support; confluent culture of differentiated Caco-2 cells; or any other type of cells capable of growing in ~~single layers~~ monolayers such as fibroblasts, tumoral, hepatic, endothelial cells, etc. Thus, examples of intestinal epithelial lines derived from tumours are Caco-2, TC7, HT29 M6; an example of kidney epithelial line is MDCK; an example of primary human skin keratinocytes is HEK; finally, examples of primary endothelian lines or cultures are HUVEC, HMEC-1, BBEC, HAEC and BAEC. Preferably, the organised two-dimensional cell culture of the invention is differentiated, polarised and is functionally active.

At page 5 of the specification, please replace the paragraph beginning at line 24 with the following replacement paragraph:

> In the method of the invention any commercial gelatine can be used, such as, for example type A pigskin gelatine. Similarly, any commercial culture medium can be used, such as, for example, DMEM (1 g/L glucose). It is "advisable" to prepare the gelatine solution a maximum of 7 days prior to applying it to the culture [[as]], otherwise, it loses part of its "preservation" properties, which are necessary for the correct functioning of the present invention.

At page 6 of the specification, please replace the paragraph beginning at line 1 with the following replacement paragraph:

> The cell culture can be prepared in the following form: firstly, performing a coating before inoculating the cells, which involves: a) positioning the inserts or *transwells* on the corresponding sized wells; 2) applying a collagen solution (or another extracellular matrix component, depending on the cell type) to the upper face of the filters (semi-permeable membranes) of each insert in DMEM culture medium (1g/L glucose) without serum (or another commercial medium); and 3) preferably leave at 37ºC in the cell cultures stove(90% humidity, 5% $CO_2$). Before using the insert, the excess coating solution is drawn up from the apical face, it is left approximately during 15 to 30 minutes in the culture stove, and the cells corresponding to the determined density ~~are determined~~ for each cell type and for each assay type are seeded. The culture is maintained for the necessary time within the scope of the functional state of the system, preferably changing the medium every 48-72 hours if necessary. The characteristics of the inserts or *transwells* used (size, pore diameter, material) are specifically determined by the cell type and the assay to which the present invention can be applied[[, in]]. In accordance with the cell type used in the culture and the assay type, controls are performed to determine the functional state of the cell system after a number of days elapsed. For example, in the case of cell systems which are used ~~are~~ as barrier models, TEER (Trans Epithelial Electric Resistance) and paracellular permeability measurements can be

used. In the case of cells systems which are used for invasion/migration assays, the migration/invasion capacity is determined by marking with a fluorochrome (e.g. calcein) [[of]] the cells which have migrated to the lower part of the filter and subsequent quantification by fluorimetry.

At page 6 of the specification, please replace the paragraph beginning at line 33 with the following replacement paragraph:

According to the present invention, the cell culture is coated with a gelatine solution in [[a]]the culture medium at a concentration of between 1 and 5 %. In general terms, the gelatine will be applied at the precise moment when it has been checked that the cell system has just reached the suitable functional state, so that the already functional cell system is immobilised, but the user has a time margin to perform their assays when the system is received. The elapsed culture time is called "lifetime". From here, the application of the present invention to ready-to-use cell systems. The lifetimes of the cell culture, i.e. the lifetime of the culture wherein the gelatine is applied, do not only depend on the culture cell types (fibroblasts, tumoral line, etc.) but also on its functional application (barrier permeability assay, adhesion assay, migration assay, invasion assay). Its determination is a question of experimental practice. Thus, in the specific case of the fibroblasts and HUVEC cells inoculated in *transwell*-type supports and in conditions wherein these cells are functionally active for a migration assay, the lifetime will be between 30 minutes and 1 hour after inoculating the cells. In the case of an invasion assay, the time will be between 1 and 24 hours. In contrast, in the case of Caco-2 cells inoculated in *transwells*, the lifetime will be 13 days after inoculating the cells, a time after which they are already functional as a barrier and the gelatine is applied, leaving the user up to day 25 of growth to perform the barrier permeability test.

At page 7 of the specification, please replace the paragraph beginning at line 29 with the following replacement paragraph:

To apply the gelatine to the culture, it is firstly necessary to completely liquefy the gelatine solution and equilibrate it to the culture medium temperature, generally at 37°C. Then, the culture medium is removed from the two compartments of each insert and the culture is washed with culture medium in the 2 compartments of each insert. Then, 2.5% liquid gelatine is applied in the apical compartment and the basal compartment, and it is left to solidify for between 2 to 3 hours in the flow hood at room temperature (20-25°C). Once the gelatine has solidified, the plates are sealed with parafilm and they are kept at room temperature until they are used (maximum of 4 days later).

At page 8 of the specification, please replace the paragraph beginning at line 5 with the following replacement paragraph:

When one wants to use the immobilised culture, the plate is incubated with solid gelatine within a cell incubator until the complete liquefication of the gelatine, preferably at 37°C, 90% humidity and 4% $CO_2$ for 3 to 4 hours until the gelatine is completely liquefied. Then, the culture is removed from both compartments ~~are eliminated~~ by suction and the culture is washed with equilibrated culture medium at 37°C. Next, the specific culture medium for the cells in question is applied and they are preferably incubated at 37°C, in 90% humidity and 5% $CO_2$ until its use.

At page 8 of the specification, please replace the paragraph beginning at line 15 with the following replacement paragraph:

In a second aspect, the invention provides a kit ~~which is used to store and/or transport~~ for storing and/or transporting the *in vitro* organized two-dimensional cell cultures according to the method of the invention, said kit comprising:

(i) an asymmetric support, and

(ii) a gelatine solution in the culture medium at a concentration of between 1 and 5%.

At page 9 of the specification, please replace the paragraph beginning at line 7 with the following replacement paragraph:

Coating: 12 hours prior to inoculating the cells, ~~position~~ the inserts (6.5 mm diameter *transwells*) are positioned on the wells of corresponding size, and ~~apply~~ a type I rat-tail collagen solution (1g/L glucose) without serum is applied to the upper face of the polycarbonate filters (0.4 µm pore diameter semi-permeable ~~filters~~ membranes) of each insert, and it is left at 37°C in the cell culture stove (90% humidity, 5% $CO_2$). Before its use, the excess coating solution is drawn up from the apical face, it is left for 15 to 30 minutes in the culture stove, and the Caco-2 cells are inoculated at a density of 5 x $10^5$ cells/$cm^2$. The culture is maintained for 13 days, changing the medium every 48-72 hours, putting 300µl of complete culture medium in the apical compartment and 900µl in the basal compartment. On day 13, ~~perform~~ the barrier state controls (polarisation) of the Caco-2 ~~single layer~~ monolayer are performed by TEER (Trans Epithelial Electric Resistance) and paracellular permeability measurements. These controls allow the functional state of the cell system to be determined as a barrier prior to coating with gelatine.

At page 10 of the specification, please replace the paragraph beginning at line 5 with the following replacement paragraph:

When one wants to use the immobilised culture, the plate is incubated with solid gelatine within a cell incubator ~~until the complete liquefication of the gelatine~~, preferably at 37°C, 90% humidity and 5% $CO_2$ for 3 to 4 hours until the gelatine is completely liquefied. Then, it is eliminated from both compartments by suction and the culture is washed with equilibrated culture medium at 37°C. Next, the specific culture medium for Caco-2 cells is applied and the cells are preferably incubated at 37°C, in 90% humidity/ 5% $CO_2$ until its use (minimum 24 hours; maximum 9 days after), changing the medium every 48-72 hours.

At page 12 of the specification, please replace the paragraph beginning at line 15 with the following replacement paragraph:

> Table II indicates some of the cell types which can be cultured in *transwell*-type supports which form ~~single layers~~ monolayers and which can be stored and transported in gelatine in its barrier state and, therefore, cultures to which the present transport method is applicable.
>
> - Caco-2, TC7, HT29 M6: intestinal epithelial lines derived from tumours.
> - MDCK: kidney epithelial lines
> - HEK: primary human skin keratinocytes
> - HUVEC, HMEC-1, BBEC, HAEC, BAEC: primary endothelial cell cultures or lines.

At page 14 of the specification, please replace the paragraph beginning at line 1 with the following replacement paragraph:

> The culture times indicated in Table II relate to the optimal time interval to obtain a polarised ~~single layer~~ monolayer, beyond which [[its]]it loses its optimum functional properties as cell barrier.

At page 14 of the specification, please replace the paragraph beginning at line 11 with the following replacement paragraph:

> The gelatine is applied to the culture at the point wherein the suitable functional state is reached. In the case of the Caco-2 cell barrier permeability test, the gelatine is preferably applied on day 13 (minimum approximate time wherein the cells begin to form a functional polarised ~~single layer~~ monolayer or barrier). They can be kept in gelatine up to approximately day 17 at room temperature and used up to approximately day 25, without losing their functional barrier properties.

At page 14 of the specification, please replace the paragraph beginning at line 20 with the following replacement paragraph:

The lifetimes of either other cell types (fibroblasts, tumoral lines) or the same described in Table II but defined for different functional applications of the barrier permeability assay (adhesion assay, migration assay, invasion assay), represent different gelatine application times. Thus, in the specific case of fibroblasts and HUVEC cells inoculated in *transwell*-type support cells and in conditions wherein these cells are functionally active for a migration assay, the lifetime will be between 30 minutes and 1 hour after inoculating the cells. In the case of an invasion assay, the time will be between 1 and 24 hours.

At page 15 of the specification, please replace the paragraph beginning at line 17 with the following replacement paragraph:

In the case of coating, 12 hours before inoculating the cells, ~~place a solution of the corresponding matrix (collagen, fibronectin, vitronectin) diluted in PBS, on the inserts (*Fluoroblock* system of 3 or 8 µm diameter) on the wells of corresponding size~~ the inserts (*Fluoroblock* system of 3 or 8µm diameter) are placed on the wells of corresponding size, a solution of the corresponding matrix (collagen, fibronectine, vitronectine, etc) diluted in PBS is applied on the upper and lower face of the fibres of each insert, and it is left at 37°C in the cell culture stove (90% humidity, 5% $CO^2$). Before its use, the excess coating solution is drawn up from the apical face, and it is left for 15 to 30 minutes in the culture stove, and the cells are inoculated at a density depending on the culture type (between $5 \times 10^4$ and $1 \times 10^5$ cells/$cm^2$). If the asymmetric support is not coated with matrix, the cells are directly inoculated on the filter. In this type of assay, during the period of cell inoculation and adhesion (between 30 minutes and 1 hour), it is not necessary to put ~~the~~ medium in the basal compartment ~~in~~ of the *Fluoroblock* system. The corresponding test is performed with part of the inoculated cells, while gelatine is applied to the rest for their maintenance at room temperature. These controls allow the functional state of the cell system to be determined.